ISSN online: 2595-7686
ISSN Impresso: 0104-9070

PAG
Pesquisa Agropecuária Gaúcha

Herbage accumulation of bahiagrass hybrids in two different environments in southern Brazil

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Abstract -The native grasslands of southern Brazil have sustained livestock farming for many years; however, decline in pasture areas has led to the loss of genetic diversity of forage species. The development of forage grass cultivars adapted to different environments, will contribute to forming new cultivated pastures or recovering degraded areas. The aim of this study was to assess the herbage accumulation of selected intraspecific hybrid progenies of bahiagrass (*P. notatum* Flügge) in two different environments (Depressão Central and Campanha region) to estimate their agronomic value. The results indicated variability for the main forage traits studied (leaf dry mass, stem dry mass, inflorescence dry mass, total dry mass, leaf: stem ratio, plant height and population density of tillers). All the hybrids producing more forage mass than the 'Pensacola' cultivar in the municipality of Eldorado do Sul and the majority also superior in the municipality of São Gabriel. The production of leaves in the forage was correlated with total dry mass (84%), suggesting that genotype selection for forage mass also selected for production of leaves. The selected hybrids should be indicated for new steps of assessment within the *P. notatum* breeding program.

Keywords: Agronomic assessment. Genetic improvement. Intraspecific hybrids. *Paspalum notatum*.

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ISSN online: 2595-7686
ISSN Impresso: 0104-9070

Acúmulo de forragem em híbridos de grama-forquilha em dois ambientes distintos no Sul do Brasil

Resumo - As pastagens nativas do Rio Grande do Sul (sul do Brasil), sustentam a pecuária há muitos anos; no entanto, o declínio em áreas de pastagens tem levado à perda da diversidade genética das espécies forrageiras e o desenvolvimento de cultivares de gramíneas forrageiras adaptadas a diferentes ambientes contribuirá para a formação de novas áresa de pastagens cultivadas ou recuperação de áreas degradadas. O objetivo deste trabalho foi avaliar o acúmulo de forragem de uma progênie híbrida intraespecífica selecionada de grama forquilha (*Paspalum notatum* Flügge) em dois ambientes distintos (Depressão Central e Campanha) para estimar seu valor agronômico. Os resultados indicaram variabilidade para as principais características forrageiras estudadas (massa seca das folhas, massa seca do caule, massa seca da inflorescência, massa seca total, relação folha: caule, altura da planta e densidade de perfilhos) com todos os híbridos produzindo mais massa de forragem do que a cultivar 'Pensacola' no município de Eldorado do Sul e a maioria também superior no município de São Gabriel. A produção de folhas foi a característica mais correlacionada com a massa seca total (84%), sugerindo que a seleção de genótipos para massa de forragem também selecionou para a produção de folhas. Os híbridos selecionados poderão ser indicados para novas etapas de avaliações dentro do programa de melhoramento de *P. notatum*.

Palavras-chave: Avaliações agronômicas. Hibridações intraespecíficas. Melhoramento genético. *Paspalum notatum*.

Introduction

The native grasslands from campos of South America represent the largest agro-ecosystem and provide valuable economic and ecosystem services (JAURENA et al., 2016). However, the lack of knowledge applied to the field, the advance of intensive agriculture and the inadequate management have led to the rapid decline of areas allocated for livestock production systems. As such, there is a need to develop new cultivars capable of mitigating the degradation of pasture ecosystems by exploring natural variability through the selection of genotypes from germplasm collections.

Several species of the genus *Paspalum* are used as forage due to its forage value and some are already being genetically improved in the United States, Brazil and Argentina to generate new *Paspalum* forage cultivars for subtropical regions (BRUGNOLI et al., 2013). *Paspalum notatum* Flügge, commonly known as 'bahiagrass', is a perennial rhizomatous grass widely distributed in Argentina, Paraguay, southern Brazil and Uruguay (QUARÍN; BURSON; BURTON, 1984). It is one of the most common species in Rio Grande do Sul State (southern Brazil) and a significant contributor to native pasture that can be used as a pioneering species to reduce the effects of soil erosion and degradation.

The native *P. notatum* germplasm is predominantly tetraploid (2n=4X=40) and apomictic (DAHMER et al., 2008) that preserve genetic diversity, but limit exploitation of the genetic potential. Artificial chromosomal duplication of sexual diploid accessions performed by Quarín et al. (2001; 2003) made it possible to match their ploidy level with apomictic genotypes, and their use in intraspecific crossbreeding programs creates new opportunities to generate hybrid plants with greater genetic variability (WEILER et al., 2015). Aguilera et al. (2011) reported that when breeding programs aim to produce cultivars with superior characteristics for a series of agronomic traits, it is vital to create synthetic cultivars from hybrid segregating populations.

Weiler et al. (2018) carried out intraspecific crosses of tetraploid *P. notatum* involving sexual genotypes called 'C4-4X', 'Q4205' and 'Q4188'(QUARÍN et al., 2001; 2003) and native apomictic ecotypes from Rio Grande do Sul State (southern Brazil), 'André da Rocha' and 'Bagual' (STEINER et al., 2017). Progeny consisting of 198 hybrid plants were established in the field by individual repetitions for agronomic assessment. Based on the results obtained, the 28 most productive plants in terms of herbage accumulation and production of leaves were selected. According to the author, ten plants were classified with erect growth habit, while 18 plants had a prostrate growth habit. Cytoembryological analyses and molecular markers were used to assess the reproduction mode of the hybrids, indicating segregation with apomictic and sexual genotypes (WEILER et al., 2017), which reinforces the possibility of producing apomictic cultivars with desirable traits and using sexual genotypes as mother plants in future crosses. The aim of this study was to evaluate the herbage accumulation of the above mentioned selected hybrid progenies and established through vegetative clones in two different environments in Rio Grande do Sul State to estimate their agronomic value through field assessments.

Materials and Methods

The experiments were conducted in Rio Grande do Sul state in the municipalities of Eldorado do Sul, in the Depressão Central region (30º06'S and 51º41'W, at an altitude of 34 m), and São Gabriel in the Campanha region (30º20'S and 54º19', at 114 m). The study was carried out from October 2012 to January 2014. The soil in the Eldorado do Sul study area is classified as typical dystrophic red argisol (ultisol) and the climate is humid subtropical (Cfa), according to Köppen's classification (1948), with hot summers. During the evaluation period, were recorded 799.6 and 720.3 mm of precipitation in the first and second year, respectively. The average temperature was around 19ºC. The soil in the São Gabriel study area is classified as red dystrophic latossolic argisol. According to Köppen's classification (1948) the climate is humid subtropical (Cfa). In evaluation period, precipitation was of 1022 mm in the first year and 726 mm in the second, and the average temperature was around 16ºC.

In both sites, soil analyses were performed before starting the experiments. Soil samples were collected at 0 to 20-cm depth, and the following characteristics are presented in Table 1. Based on the soil analysis, the management and fertilization procedures adopted were in line with the technical recommendations for summer perennial grasses of the Soil Chemistry and Fertility Commission (CQFS, 2004).

The 28 hybrids genotypes of *P. notatum* studied were: 18 apomicts (A16, A20, B17, B26, B29, B35, B37, B43, C2, C6, C9, C15, C22, C17, C23, D3, F15, F24 and F39) and ten sexuals (B2, B28, C18, C24, C32, D16, D17, D23 and D25) from an intraspecific crosses selected by Weiler et al. (2018): 'Q4188 × André da Rocha' (progeny A), 'Q4188' × 'Bagual' (progeny B), 'Q4205' × 'André da Rocha' (progeny C),

ISSN online: 2595-7686
ISSN Impresso: 0104-9070

‘Q4205’ × ‘Bagual’ (progeny D) and ‘C44X’ × ‘Bagual’ (progeny F). *P. notatum* cv. ‘Pensacola’ was used as a control because it is one of the few alternatives of *Paspalum* species that has seeds commercially available and the ‘Baio’ ecotype too was used as a control due to its known forage potential when compared to the other ecotypes tested (STEINER et al., 2017). The parents also were evaluated.

In the fall 2012, the 34 selected genotypes were clonally propagated in the field of the Agronomic Experimental Station of the UFRGS. Through the tillers, 64 seedlings of each genotype were made which remained in a greenhouse during the winter. The transplanting of the seedlings to the field occurred during October and December 2012 in Eldorado do Sul and São Gabriel, respectively.

Table 1. Chemical characteristics of soil in Eldorado do Sul (1) and São Gabriel (2) before starting the experiment.

Site	Clay	pH H_2O	OM	P	K	H+Al	Al	Ca	Mg	CEC	Base sat.	Al sat.
	g kg^{-1}		g kg^{-1}	mg dm^{-3}		cmol$_c$ dm^{-3}					%	
1	260	4,7	21,0	6,7	113	7,7	0,5	4,7	0,5	10,2	24	16,7
2	260	5,8	35,0	2,2	104	4,4	0,0	9,7	4,7	14,7	77	0,0

In a randomized complete block design was used with three replications, and the experimental units consisted of two-meter-long rows with one genotype each. Each row contained nine plants and assessments were performed by harvest in the central meter of each row. Due to different growth habit classified by Weiler et al. (2018), the following evaluation criterion was adopted: plants with erect growth habit were harvested when they reached 30 cm height, while plants with prostrate growth habit were harvested at 20 cm, leaving 5 cm stubble height. The harvest with scissor was carried out when around 80% of the genotypes (erect and prostate) reached the canopy height established.

In both sites, four harvest were performed in the first year and three in the second. In each harvest, the samples were separated into leaves, stems and inflorescences and dried in a air-forced drier oven at 55ºC until constant weight. The traits assessed were: leaf dry mass (LDM, g row^{-1}), stem dry mass (SDM, g row^{-1}), inflorescence dry mass (IDM, g row^{-1}), total dry mass (TDM, g row^{-1}) and leaf: stem ratio (LSR). Plant height (PH, cm) was measured from the soil up to height of the leaves. Population density of tillers (PDT) was accessed using three squares (0.25 x 0.25 m) per row. The variable total dry mass was used as a criterion for genotype selection, while the variables leaf dry mass, stem dry mass, inflorescence dry mass, total dry mass and population density of tillers were evaluated with the purpose of performing the correlation analysis.

The forage mass data collected were submitted to analysis of variance using the PROC GLM procedure in SAS statistical software (SAS, 2001). When differences between means were significant, Dunnett’s test was performed at 5% probability to compare with two controls: ‘Bagual’ ecotype and ‘Pensacola’ cultivar. Pearson’s correlation coefficient was used to test the associates among all aforementioned variables.

ISSN online: 2595-7686
ISSN Impresso: 0104-9070

Results and Discussion

The means obtained for total dry mass indicated variability generated by intraspecific hybridization in both locations. Other authors have reported significant variability for different agronomic traits in the genus *Paspalum.* A high level of diversity was recorded for a series of agronomic and morphological characteristics in intraspecific segregating progeny of *P. notatum* (ZILLI et al., 2015) and in interspecific hybrid progeny obtained by crossbreeding with different species of the Plicatula group of the genus (NOVO et al., 2017). Pereira et al. (2012) reported genetic variability and the expression of traits of interest for forage production in genotypes of *P. lepton* (ex- *P. nicorae*) and *P. guenoarum,* and Brugnoli et al. (2013) in *P. simplex* populations.

Table 2. Total dry mass (TDM, g row^{-1}) of the genotype per year and overall in the municipality of Eldorado do Sul.

	Year 1												Year 2									
	12/08/2012			01/18/2013			02/16/2013			03/22/2013			10/28/2013			12/06/2013			01/06/2014			Total
GEN	TDM	B	P	TDM	B	P	TDM	B	P	TDM	B	P	TDM	B	P	TDM	B	P	TDM	B	P	TDM
B43	87.8			306.5		*	133.9		*	196.2		*	217.1	*	*	252.6		*	339.4		*	1533.5
C9	69.0			286.0			128.6			189.1		*	157.6	*	*	298.4	*	*	302.8		*	1431.6
B26	92.4		*	359.1	*	*	157.9		*	253.9	*	*	169.4	*	*	182.3			187.4			1402.3
C18	109.0		*	296.9		*	131.6			239.2	*	*	123.3			261.3		*	229.7			1391.0
B17	68.9			241.2			132.0			202.2		*	174.6	*	*	284.6	*	*	251.9			1355.4
C22	85.0			285.4			121.9			188.4		*	166.5	*	*	289.2	*	*	202.8			1339.2
D25	131.6	*	*	299.0		*	128.0			205.8	*	*	195.1	*	*	177.6			196.0			1333.1
C15	105.4		*	280.7			107.3			180.7		*	181.0	*	*	244.7			229.7			1329.5
D16	98.6		*	249.5			118.9			170.3		*	131.2		*	223.3			303.2		*	1295.0
A16	79.8			235.3			161.6		*	183.8		*	174.0	*	*	220.8			233.8			1289.2
C24	76.2			232.4			128.1			143.7		*	95.1			269.5		*	329.1		*	1274.0
B29	64.5			271.0			134.3		*	201.7		*	203.4	*	*	230.9			161.4	*		1267.1
C32	67.4			304.7		*	124.7			210.3	*	*	130.5	*	*	231.0			193.2			1261.8
F15	70.0			274.7			116.5			184.9		*	75.7			184.9			314.9		*	1221.6
D23	72.7			230.6			108.9			138.8		*	109.3			251.0		*	308.6		*	1219.9
B28	97.1		*	295.7		*	176.6		*	243.1	*	*	149.3	*	*	134.8			111.6	*		1208.1
B2	84.2			257.1			136.7		*	167.3		*	160.5	*	*	215.0			129.0	*		1149.7
B35	58.0			188.1			115.2			162.8		*	146.3	*	*	202.5			259.0			1131.9
C17	77.7			246.5			114.5			139.2		*	157.0	*	*	199.4			188.4			1122.6
B37	69.4			230.5			108.6			134.6		*	184.9	*	*	196.8			180.5			1105.3
Q4188	59.6			228.2			118.3			162.3		*	103.7			194.7			200.8			1067.5
D17	64.7			208.8			108.9			116.4		*	112.0			216.1			237.5			1064.5
Bag	68.3			193.6			119.8			156.5		*	58.3			138.5			285.1			1020.0
AR	37.8			137.9			119.5			148.2		*	69.6			155.8			330.6		*	999.4
C6	58.7			176.6			99.6			110.8			97.5			216.3			217.4			976.9
D3	45.6			168.7			102.2			188.3		*	118.1			158.8			191.4			973.2
C2	70.4			226.8			112.1			122.6		*	105.8			118.4			212.6			968.7
F24	39.4			146.1			100.3			149.3		*	58.5			193.5			274.5			961.5
F39	37.4			166.6			101.3			125.3		*	55.5			139.0			293.5			918.7
A20	45.8			178.7			103.7			97.3	*		84.9			174.5			179.9			864.8
C23	58.7			177.5			92.4			181.8		*	47.0			59.9			135.3	*		752.6
P	35.4			167.2			70.5			65.4	*		46.0			172.6			178.4			735.5
Q4205	74.2			196.6			94.1			108.1			71.9			80.4			103.2	*		728.5
C4-4X	15.8	*		76.7			67.4			98.9	*		39.9			55.2			58.1	*		412.0

(*) values significant at $p \leq 0.05$, compared to the controls 'Bagual' (B) and 'Pensacola' (P) per cutting. Genotype (GEN); Parents: 'Q4250', 'Q4188', 'C4-4X' (female) and 'André da Rocha' (AR), 'Bagual' (B) (male).

In Eldorado do Sul, the best mother plant ('Q4188') produced forage mass around 655 g row^{-1} more than 'C4-4X', the least productive mother plant (Table 2). This result can be attributed to different growth habit, since the 'Q4188'plant exhibit an erect habit, unlike the 'C4-4X' plant, which exhibit a prostrate habit. Since the forage was collected 5 cm above the ground, a significant portion of the prostrate plants was not harvested, resulting in lower total dry mass values. These data suggest that the least productive mother plant should be replaced by another sexual genotype in future crosses when the selection criterion is herbage accumulation. The hybrids too were compared with the most productive male parent (‘Bagual’) and some hybrids were more productive from the fourth harvest (March 2013). However, B26 was more productive in second harvest (January 2013).

In a comparison with cv. ‘Pensacola’ at Eldorado do Sul, hybrids had greater herbage accumulation for all the harvest performed with hybrid B43 producing almost two times more than the cultivar. From the second year, in fifth harvest (October 2013) onwards this genotype was among the most productive, demonstrating good post-winter early regrowth capacity. Selecting genotypes with rapid growth of summer is important because it may provide earlier grazing. Acuña et al. (2009, 2011) studied the first and second generations of intraspecific *P. notatum* hybrids and found that, in addition to variability for several morphological characteristics, the hybrids also showed high regrowth capacity in fall and spring and cold stress tolerance.

According to Melo et al. (2007), identifying cultivars with higher yield stability is a widely used alternative to minimize the effects of genotype interaction with the environment and make the cultivar selection process more reliable. The higher herbage accumulation in the second year is common in perennial species, since they generally yield less during establishment (PEREIRA et al., 2012; MOTTA et al., 2017). This demonstrates that selecting superior genotypes of perennial forage species should not be based solely on herbage accumulation recorded in the first year.

In the municipality of São Gabriel (Table 3), there was no significant difference between hybrids, parents and controls in herbage accumulation. However, differences between hybrids were more pronounced at the sixth and seventh harvests: B17, C6, C9 and B2 maintained high herbage accumulation in both years, indicating adequate yield stability at this site. Hybrids C9, C18 and B17 were among the ten most productive at both sites. The hybrid B17 displayed approximately 65% higher forage mass (372 g row^{-1}) when compared to ‘Pensacola’. ‘C4-4X’ was less productive than the genotypes ‘Q4188’ and ‘Q4205’, confirming the low herbage accumulation of that mother plant. Motta et al. (2017) reported superior agronomic potential and high cold tolerance in interspecific *P. plicatulum x P. guenoarum* hybrids when compared with their parent plant.

Most breeding programs aim for varieties that perform well across different environments (FÈ et al., 2015). This is an important trait in forage breeding programs, since according to Piana; Silva; Antunes (2012), variations resulting from different environments are obstacle to obtaining genotypes that main superior and consistently higher herbage accumulation in different locations of a large area. The opposite behavior was observed in genotype B43, which was most productive across the experimental period in Eldorado do Sul, but least productive in São Gabriel, suggesting that this hybrid should be indicated only for favorable environments to its growing.

The higher herbage accumulation in the second year is common in perennial species, which generally yield less during establishment (PEREIRA et al., 2012). This demonstrates that selecting superior

ISSN online: 2595-7686
ISSN Impresso: 0104-9070

genotypes of perennial forage species should not be based solely on forage mass recorded in the first year. Fè et al. (2015) estimated the total amount of genetic and environmental variance for important traits in the breeding program of *Lolium perenne* (perennial ryegrass) and observed that some families produced more in the first year and less in the second year and vice versa. According to Temesgen et al. (2015), yield and stability should be considered simultaneously to explore the effects of genotype interaction with the environment and to make the selection of favorable genotypes more precise.

Table 3. Total dry mass (TDM, g row^{-1}) of the genotypes per cutting and overall in the municipality of São Gabriel.

	Year 1												Year 2									
	01/24/2013			04/08/2013			03/05/2013			04/08/2013			10/15/2013			11/20/2013			12/16/2013			Total
GEN	TDM	B	P	TDM	B	P	TDM	B	P	TDM	B	P	TDM	B	P	TDM	B	P	TDM	B	P	TDM
B17	51.7			53.4			54.3			60.2			184.6		*	301.7	*	*	238.7			944.4
B37	62.8			52.4			41.7			75.1			126.9			214.8			188.0			761.7
D3	54.2			40.5			33.7			42.1			113.5			250.4			215.6			749.9
F24	66.7			51.9			55.0			44.5			94.7			163.4			242.4			718.5
C9	71.7			51.0			57.6			39.5			138.7			186.6			169.2			714.3
C6	60.6			42.7			52.1			46.7			126.9			184.5			186.9			700.4
B2	84.7			41.7			52.9			30.1			105.6			185.2			200.1			700.3
C32	42.2			41.8			32.7			70.5			84.4			208.2			217.9			697.6
C18	66.9			48.1			46.3			61.6			102.6			171.3			191.1			687.9
Bag	64.7			49.9			66.8			67.1			99.7			160.0			167.4			675.5
D25	65.5			51.8			57.0			44.6			88.9			161.1			197.8			666.8
B26	61.1			41.5			42.4			45.6			110.4			189.5			159.2			649.9
B28	62.0			49.0			51.8			60.3			82.1			198.9			141.0			645.2
F15	53.2			29.9			34.4			71.9			78.5			162.8			214.2			644.9
A16	63.3			48.4			49.7			74.1			107.6			106.6			189.0			638.7
B29	59.5			47.8			44.0			58.8			91.4			186.3			135.0			622.9
C2	65.8			42.5			33.4			50.3			86.4			172.4			172.0			622.8
C17	48.9			42.4			33.9			39.2			82.5			200.9			169.2			617.1
C22	46.3			40.1			31.0			50.2			68.4			210.0			165.3			611.3
B35	52.7			39.6			41.6			41.2			93.3			162.0			174.5			605.0
A20	59.9			39.8			38.2			45.1			85.2			155.3			174.0			597.5
C24	50.0			63.1			36.7			55.0			79.1			123.6			189.7			597.2
D16	58.2			42.9			44.1			74.3			61.9			141.4			155.9			578.6
C15	69.1			37.8			38.0			31.6			89.4			158.4			152.9			577.1
P	54.5			40.0			55.3			51.0			72.4			141.6			157.7			572.4
D17	55.1			37.1			27.9			61.4			46.6			154.6			183.8			566.5
F39	36.5			37.1			32.5			49.8			55.8			138.0			191.7			541.3
AR	41.6			43.6			37.4			54.6			68.3			106.1			176.8			528.3
Q4205	59.4			50.7			40.7			68.7			83.5			118.9			93.8			515.5
C23	51.4			36.7			31.3			69.7			61.5			106.7			155.0			512.2
D23	75.8			36.9			42.2			46.8			68.8			113.2			116.7			500.3
B43	52.9			39.9			36.6			47.4			85.9			77.2			158.2			498.1
Q4188	51.4			39.7			35.9			48.7			79.2			106.7			106.8			468.3
C4-4X	58.4			38.9			34.1			45.5			81.2			32.2	*		39.3	*	*	329.6

(*) values significant at $p \leq 0.05$, compared to the controls 'Bagual' (B) and 'Pensacola' (P) per cutting. Genotype (GEN); Parents: 'Q4250', 'Q4188', 'C4-4X' (female) and 'André da Rocha' (AR), 'Bagual' (B) (male).

The variables leaf dry mass, stem dry mass and inflorescence dry mass showed a positive and significant correlation with total dry mass (Table 4). Other authors also reported correlations between total dry mass and leaf dry mass in forage species such as *Penisetum purpureum* (SILVA et al., 2008), *Brachiaria brizantha* (BASSO et al., 2009), *Brachiaria ruziziensis* (BORGES et al., 2011), hybrids of *Paspalum plicatulum* x *P. guenoarum* (MOTTA et al., 2016) and hybrids of *Paspalum notatum* (WEILER et al., 2018). Our findings suggest that there is no need to separate yield components, i.e. genotype selection by total dry mass also will be selecting genotypes with higher leaf dry mass and inflorescence dry mass. These traits are important for selection of forage plants, since it the leaf has greater nutritive value than other components, while greater inflorescence dry mass could be indicating a greater seed production. As such, this strategy enables faster assessment, particularly when a large number of accessions/genotype are tested.

Table 4. Coefficients of phenotypic correlation for traits linked to herbage accumulation (Total Dry Mass) in *P. notatum* hybrids studied in two different environments.

Trait	TDM	LDM	SDM	IDM	PDT
LDM	0.84*				
SDM	0.79*	0.36*			
IDM	0.76*	0.42*	0.84*		
PDT	0.23*	0.08	0.02	0.07	
PH	0.60*	0.49*	0.48*	0.39*	0.33*

(*) values significant at $p \leq 0.05$; TDM – total dry mass; LDM – leaf dry mass; SDM – stem dry mass; IDM – inflorescence dry mass; PDT – population density of tillers; PH – plant height.

The population density of tillers displayed a weak correlation with total dry mass, indicating that tiller density alone does not explain the amount of total dry mass produced, which is also influenced by the number of leavers per tiller. This weak correlation was unexpected but can be explained by the growth habit of *P. notatum,* which invests a significant portion of assimilates in reserve structures (rhizomes) in initial years, which are analyzed when the plant is cut. The plant height was significantly and moderately correlated with total dry mass. This moderately association can be explained by the different growth habit among genotypes, since it prostate plants shown lower height than erect plants.

Conclusion

The results confirm significant variability on herbage accumulation of the tetraploid hybrids studied. The apomictic hybrids B17, B26, B37, B43, C6, C9, C15, C22, D3 and F24 and the sexuals hybrids B2, C18, C32 and D25 showed more forage mass than 'Pensacola' and the best male parent plant and should be indicated for new steps of assessment within the *P. notatum* breeding program.

Acknowledgements

The authors thank the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil, Edital Universal 2010, proc. 471318/2010-8**)** and South-Brazilian Association for the Promotion of Forage (Sulpasto) for financial support and grants.

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